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Vasorelaxant effects of 1-nitro-2-phenylethane, the main constituent of the essential oil of *Aniba canelilla*, in superior mesenteric arteries from spontaneously hypertensive rats

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ABSTRACT

The present study investigated the mechanisms underlying the vasorelaxant effects of the essential oil of *Aniba canelilla* (EOAC) and its main constituent 1-nitro-2-phenylethane (NP) in isolated superior mesenteric artery from spontaneously hypertensive rats (SHRs). At 0.1–1000 μg/mL, EOAC and NP relaxed SMA preparations pre-contracted with 75 mM KCl with IC_{50} (geometric mean [95% confidence interval]) values of 294.19 [158.20–94.64] and 501.27 [378.60–624.00] μg/mL, respectively); or with phenylephrine (PHE) (IC_{50}s = 11.07 [6.40–15.68] and 7.91 [4.08–11.74) μg/mL, respectively). All these effects were reversible and remained unaltered by vascular endothelium removal. In preparations maintained under Ca^{2+}-free conditions, EOAC and NP (both at 600 μg/mL) reduced the PHE-, but not the caffeine-induced contraction. In Ca^{2+}-free and high K^+ (75 mM) medium, the contractions produced by $CaCl_2$ or $BaCl_2$ were reduced or even abolished by EOAC and NP at 100 and 600 μg/mL, respectively. EOAC and NP (both at 10–1000 μg/mL) also relaxed the contraction evoked by phorbol dibutyrate (IC_{50} = 52.66 [10.82–94.64] and 39.13 [31.55–46.72] μg/mL, respectively). It is concluded that NP has a myogenic endothelium-independent vasorelaxant effects and appears to be the active principle of the EOAC. Vasorelaxant effect induced by both EOAC and NP is preferential to receptor-activated pathways and it appears to occur intracellularly more than a superficial action restricted to the membrane environment such as a simple blocking activity on a given receptor or ion channel.

1. Introduction

Aniba canelilla (H.B.K.) Mez [syn. *Aniba elliptica* A. C. Sm., *Cryptocarya canelilla* Kunth], (Lauraceae) is an aromatic plant abundant in the Amazon region, where it is commonly known as "casca-preciosa" (precious bark). This plant is an important and historically interesting species in the Amazon forest because it was confused with cinnamon trees (*Cinnamomum zeylanicum* Blume) during the 1540 voyage of Pizarro and Orellana from the Andes to the Amazon estuary and during Humbolt and Bonpland's 1800 expedition in the Orinoco River basin to find the "famous cinnamon" (Naranjo and Kijjoa, 1981).

The trunk wood, fine stems and leaves of casca-preciosa are used as spices and ingredients for local dishes, fragrances, and sachets. In folk medicine, decoctions of bark from *A. canelilla* are commonly used for their antispasmodic, digestive stimulating and carminative properties (Maia et al., 2001). Stem bark of *A. canelilla* has an essential oil content of 1% by dry weight. The odoriferous principle of leaf, bark, and trunk wood of *A. canelilla* is 1-nitro-2-phenylethane (NP) (Fig. 1), also responsible for the plant's cinnamon scent (Gottlieb and Magalhães, 1960). Methyleugenol is also an other important volatile constituent of the essential oil of *A. canelilla* (EOAC) (Gottlieb and Magalhães, 1960). NP is considered to be a major contributor to tomato flavor, as are 2-phenylacetaldehyde and 2-phenylethanol (Tieman et al., 2006). Nitroderivatives found in higher plants are rare. In the case of

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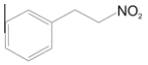

Fig. 1. Chemical structure of 1-nitro-2-phenylethane.

NP, its biogenesis involves phenylalanine oxidation followed by spontaneous decarboxylation (Gottlieb et al., 1961). Oger and co-workers reported fungistatic properties for bark EOAC (Oger et al., 1994). Furthermore, it has been reported that bark EOAC exerts relaxant effects on intestinal smooth muscle, supporting the use of the plant in folk medicine for the treatment of gastrointestinal disorders (Maia et al., 2003). Recently, antioxidant activity of the EOAC and NP (da Silva et al., 2007) as well as antinociceptive activity of NP (de Lima et al., 2009) have been reported.

Recently, we showed that intravenous (i.v.) administration of EOAC and its main constituent NP induced two periods of hypotension and bradycardia in anesthetized, spontaneously hypertensive rats (SHRs). Initially a rapid bradycardia (onset time of 1–2 s) occurred coincidentally (onset time of 2 s) with an arterial hypotension (phase 1) and then, a delayed decrease in blood pressure associated with a second bradycardia (phase 2) (Interaminense et al., 2011). Several lines of evidence confirmed that phase 1 bradycardiac and depressor responses to EOAC and NP was of reflex origin (vago-vagal reflex), that apparently resulted from the stimulation of vagal pulmonary rather than cardiac C-fiber afferents. The transduction mechanism of the EOAC and NP excitation of C-fiber endings is not fully understood and seems not to involve the activation of either vanilloid $TPRV_1$ or 5-HT_3 receptors located on vagal sensory nerves (Interaminense et al., 2011). The second hypotensive response (phase 2) to i.v. EOAC and NP seems resulting, at least in part, from their direct vasodilatory effect on the peripheral smooth muscle (Interaminense et al., 2011). These *in vitro* and *in vivo* effects have been also observed with NP in normotensive rats (de Siqueira et al., 2010). Therefore, the present study investigated the mechanism(s) underlying the vasorelaxant effects of NP in isolated superior mesenteric artery (SMA) preparations from SHRs, and compared it to that of EOAC.

2. Materials and Methods

2.1. Plant material

Barks of *A. canelilla* were collected in May, 2005, in the area of Cauaxi River, Municipality of Paragominas, southeast of Pará state, Brazil. Its identification was confirmed by Dr. da Silva M.H.L. (Department of Botanic, Emílio Goeldi Museum, Belém, Pará, Brazil). A voucher specimen (#174904) is deposited in the herbarium of Emílio Goeldi Museum, in the city of Belém, Pará State, Brazil.

2.2. Essential oil distillation, fractionation and analysis

Bark was air-dried, grinded and submitted to hydrodistillation (100 g, 4 h) using a Clevenger-type apparatus for EOAC obtention. Analytical conditions, composition of the EOAC used in the present study and retention indices of its constituents have been previously reported ([Interaminense et al., 2011] and [Lahlou et al., 2005]). Briefly, the sample of EOAC used in the present study contained 52.4% of NP, 38.6% of methyleugenol and other minor constituents ([Interaminense et al., 2011] and [Lahlou et al., 2005]).

In order to purify the main constituent NP, bark essential oil (15 g) was submitted to fractionation in a silica gel chromatographic column using petroleum ether (isocratic elution) and thin-layer chromatography (hexane–ethyl acetate, 9:1). The percentage content of NP in the oil and in the purified fractions was obtained in a Thermo Focus GC/FID operated under the following conditions: WCOT DB-5 ms (30 m x 0.25 mm; 0.25 μm film thickness) fused silica capillary column; temperature programmed, 60–240 °C (3 °C/min); injector and detector temperatures, 220 and 250 °C; carrier gas, nitrogen; injection type, splitless (2 μL, of a 1:1000 hexane solution). The ^{1}H NMR spectrum was obtained in a Varian Mercury NMR at 300 MHz using $CDCl_3$ as solvent.

2.3. Solutions and drugs

EOAC and NP were first dissolved in dimethyl sulfoxide (DMSO, Sigma Chemicals Co., St Louis, MO, USA) up to 1% of the total volume, made up with the perfusion medium and sonicated just before use. The perfusion medium used was fresh Krebs-Henseleit solution (KHS) buffer (pH 7.4) of the following composition (in mM): NaCl 118; KCl 4.7; $NaHCO_3$ 25; $CaCl_2.2H_2O$ 2.5; KH_2PO_4 1.2; $MgSO_4.7H_2O$ 1.2; glucose 11 and ethylenediaminetetraacetic acid (EDTA) 0.01. In some experiments, a modified KHS's solution containing equimolar $BaCl_2$, instead of $CaCl_2$ was used. Phenylephrine (PHE) hydrochloride, acetylcholine chloride, phorbol 12, 13-dibutyrate (PDB), tetraethylammonium (TEA) chloride, noradrenaline (NA), caffeine and nifedipine hydrochloride, which were all purchased from Sigma, were first dissolved in distilled water and were made up with KHS.

2.4. Animals

Adult male SHRs (age: 16–18 weeks) were obtained from local colonies maintained at the Department of Physiology and Pharmacology, Federal University of Pernambuco, Recife, Brazil. They were kept under conditions of constant temperature (22 ± 2 °C) with a 12 h light/12 h dark cycle and free access to food and water. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication 85–23, revised 1996). All procedures described here were reviewed by and had prior approval from local animal ethics committee (23076.038710/2010–12).

2.5. Tissues preparation and experimental protocols

Rats were sacrificed by cervical dislocation. For isometric tension recording, SMA was removed and placed in cold oxygenated KHS buffer. Segments of SMA (3 mm in length), free of fat and connective tissue, were mounted between two steel hooks in isolated tissue chambers containing gassed (95% O_2 and 5% CO_2) KHS, at 37 °C, under a resting tension of 0.5 g (optimal resting tension), which was readjusted every 15 min during a 45-min equilibration period before drug administration. Isometric tension was recorded by using an isometric force displacement transducer (Letica TRI 201, Panlab, S.L., Barcelona, Spain) connected to an acquisition system (ML870/P ADInstruments Pty Ltd, Castle Hill, Australia). Vessels were initially exposed twice to 75 mM KCl to check their functional integrity. After 30 min, rings were contracted with a concentration of PHE inducing 50–70% of the contraction induced by KCl and acetylcholine (1 μM) was then added to assess endothelium integrity. Sixty min later, the series of experiments 1 to 7 were performed.

2.5.1. Series 1

This series of experiments were carried out to assess the effects of EOAC (0.1–1000 μg/mL) and NP (0.1–1000 μg/mL) on the spontaneous tone of endothelium-containing SMA preparations. In